

PCT/EP98/06298
REIMANN, Hansjörg et al.
Our Ref.: C 2086 PCT

Claims

1. A polynucleotide encoding a fusion protein which is stable in a cell, said fusion protein comprising:
 - (a) a first (poly)peptide which as such is unstable in a cell; and
 - (b) a second (poly)peptide which co-precipitates a chaperone.
2. The polynucleotide of claim 1, wherein said first (poly)peptide is or comprises an epitope and/or a functional and/or structural domain of a protein, and/or a mutated or truncated variant of a protein.
3. The polynucleotide of claims 1 or 2, wherein said chaperone belongs to the family of heat shock protein (hsp)70 chaperones.
4. The polynucleotide of claim 3, wherein said chaperone is hsp73.
5. The polynucleotide of any one of claims 1 to 4, wherein said second (poly)peptide is a viral T antigen carrying an internal and/or C-terminal deletion.
6. A polynucleotide encoding a fusion protein which is stable in a cell, said fusion protein comprising a first (poly)peptide and a second (poly)peptide which is a viral T antigen carrying an internal and/or C-terminal deletion.
7. The polynucleotide of claim 5 or 6, wherein the function and/or structure of the N-terminal J domain of said viral T antigen is maintained.
8. The polynucleotide of any one of claims 5 to 7, wherein said viral T antigen is a viral large T antigen.
9. The polynucleotide of any one of claims 5 to 7, wherein said viral T antigen is SV40 T antigen.

10. The polynucleotide of claim 8, wherein said viral large T antigen is SV40 large T antigen.
11. The polynucleotide of claim 10, wherein the about 300 C-terminal amino acids of said SV40 large T antigen are deleted.
12. The polynucleotide of claim 10 or 11, wherein said SV40 large T antigen contains amino acids 1 to 272.
13. The polynucleotide of any one of claims 10 to 12, wherein the internal deletion comprises at least part of the nuclear localisation signal.
14. The polynucleotide of claim 13, wherein amino acids 110 to 152 are deleted.
15. The polynucleotide of any one of claims 1 to 14 further encoding a tag.
16. The polynucleotide of any one of claims 1 to 15, wherein said first and second (poly)peptide are linked via a protease cleavage site.
17. A vector comprising the polynucleotide of any one of claims 1 to 16.
18. The vector of claim 17, wherein said polynucleotide is operatively linked to an expression control sequence.
19. A host cell comprising the polynucleotide of any one of claims 1 to 16, or the vector of claim 17 or 18.
20. The host cell of claim 19 which is a eukaryotic or prokaryotic cell.
21. A method for the production of the fusion protein as defined in any one of claims 1 to 16, said method comprising:
- (a) culturing the host cell of claim 19 or 20 under conditions that allow the synthesis of said fusion protein; and

(b) recovering said fusion protein from the culture.

22. The method of claim 21 further comprising the step of separating said fusion protein from complexed chaperones.
23. A fusion protein encoded by the polynucleotide of any one of claims 1 to 16, or the vector of claim 17 or 18, or obtainable or obtained by the method of claim 21 or 22.
24. A method for the production of a first (poly)peptide as defined in claim 1 or 2, said method comprising:
- (a) culturing the host cell of claim 19 or 20 under conditions that allow the synthesis of a fusion protein as defined in claim 16;
 - (b) recovering said fusion protein from the culture; and
 - (c) separating said second (poly)peptide from said fusion protein by proteolytic cleavage.
25. A method for the production of a complex comprising the fusion protein of claim 23 and a chaperone as defined in claim 4 or 5, said method comprising:
- (a) culturing the host cell of claim 19 or 20 under conditions that allow complex formation of said fusion protein with said chaperone; and
 - (b) recovering said complex from the culture.
26. A method for the production of an antibody directed against a first (poly)peptide as defined in claim 1 or 2, said method comprising administering in an amount sufficient to induce a humoral immune response the polynucleotide of any one of claims 1 to 16, the vector of claim 17 or 18, the fusion protein of claim 23, the first (poly)peptide obtainable or obtained by the method of claim 24, and/or the complex obtainable or obtained by the method of claim 25 to a subject.
27. A method of immunizing a subject, said method comprising administering in an amount sufficient to induce a humoral and/or cellular immune response the polynucleotide of any one of claims 1 to 16, the vector of claim 17 or 18, the fusion protein of claim 23, the first (poly)peptide obtainable or obtained by the

method of claim 24, and/or the complex obtainable or obtained by the method of claim 25 to said subject.

28. A kit comprising:

- (a) the polynucleotide of any one of claims 1 to 16;
- (b) the vector of claim 17 or 18;
- (c) the host cell of claim 19 or 20;
- (d) the fusion protein of claim 23;
- (e) the first (poly)peptide obtainable or obtained by the method of claim 24;
- (f) the complex obtainable or obtained by the method of claim 25; and/or
- (g) the antibody obtainable or obtained by the method of claim 26.

29. A diagnostic composition comprising the polynucleotide of any one of claims 1 to 16, the vector of claim 17 or 18, the fusion protein of claim 23, the first (poly)peptide obtainable or obtained by the method of claim 24, the complex obtainable or obtained by the method of claim 25, and/or the antibody obtainable or obtained by the method of claim 26.

30. A method for the detection of the presence of an epitope comprised in a (poly)peptide as defined in claim 1 or 2, said method comprising:

- (a) contacting the fusion protein of claim 23 or the first (poly)peptide obtainable or obtained by the method of claim 24 with an antibody or a cytotoxic T-lymphocyte (CTL) under conditions that allow binding of said antibody or CTL to said epitope; and
- (b) detecting whether the antibody or CTL has bound to said epitope.

31. The method of claim 30, wherein said antibody or CTL is derived from an individual infected with a pathogen.

32. The method of claim 30 or 31, wherein the first (poly)peptide of said fusion protein or said first (poly)peptide obtainable or obtained by the method of claim 24 is derived from a pathogen.

33. A pharmaceutical composition comprising the polynucleotide of any one of claims 1 to 16, the vector of claim 17 or 18, the fusion protein of claim 23, the first (poly)peptide obtainable or obtained by the method of claim 24, the complex obtainable or obtained by the method of claim 25, and/or the antibody obtainable or obtained by the method of claim 26, and, optionally, a pharmaceutically acceptable carrier and/or diluent.
34. The pharmaceutical composition of claim 33 which is a vaccine.
35. The pharmaceutical composition of claim 34, wherein said vaccine induces a humoral and/or cellular immune response.
36. Use of the polynucleotide of any one of claims 1 to 16 or the vector of claim 17 or 18 for the production of an antibody directed against a first (poly)peptide as defined in claim 1 or 2.
37. Use of a (poly)peptide comprising an epitope detected by the method of any one of claims 30 to 32 or a complex produced by the method of claim 25 for the production of an antibody.